1973

PASADENA
CALIFORNIA

CITRUS RESEARCH CONFERENCE

CITRUS RESEARCH CONFERENCE

December 4, 1973

Pasadena, California

ABSTRACTS OF PAPERS

Sponsored By:

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California 91106

Southern California-Hawaii Area, Western Region
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by the Agricultural Research Service, U.S. Department of Agriculture. The following are participating in this year's conference.

Western Region

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, California 91106

Western Regional Research Center
Berkeley, California 94710

Southern Region

Citrus and Subtropical Products Laboratory
600 Avenue S, N.W., Winter Haven, Florida 33882

Food Crops Utilization Research Laboratory
P.O. Box 388, Weslaco, Texas 78596

Conference headquarters:

Huntington-Sheraton Hotel
1401 South Oak Knoll Avenue
Pasadena, California

P R O G R A M

CITRUS RESEARCH CONFERENCE

Tuesday, December 4, 1973

MORNING SESSION - 9:00 A.M.

Abstract on page

WELCOME: S. E. Jones, Area Director, Southern California-Hawaii Area, ARS/USDA, Riverside, California

INTRODUCTORY REMARKS: H. Rex Thomas, Deputy Administrator, Western Region, ARS/USDA, Berkeley, California

CHAIRMAN: V. P. Maier, Chief, Fruit and Vegetable Chemistry Laboratory, Pasadena, California

L U N C H

AFTERNOON SESSION - 1:45 P.M.

A NEW APPROACH TO NICOTINIC ACID DETERMINATION IN CITRUS JUICES*

Carl E. Vandercook and Ruth L. Price
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Due to the Food and Drug Administration's current emphasis and future requirements for nutritional labeling, there is a great deal of activity in the field of vitamin analysis. A real need exists to improve the sensitivity, accuracy, and especially the speed of vitamin assay methods. We plan to investigate vitamin assay methods for citrus juices and other fruit products, with the goal of automating as many of the procedures as possible.

Although ascorbic acid is of major importance in orange juice, there are other vitamins present in relatively small amounts. These minor vitamins are present in amounts roughly equivalent to the RDA per Calorie of juice. Nicotinic acid, as one of the minor vitamins, has the potential for a dual application. In addition to its nutritional aspects, it has been suggested as a possible indicator of juice content. Unfortunately, orange juice does not lend itself to the Association of Official Analytical Chemists (AOAC) standard chemical method of nicotinic acid analysis. The acid hydrolysis step turns the juice a dark brown, which obscures the faint yellow produced in the subsequent reaction. The alternative microbiological method leaves much to be desired as a routine method. Consequently, research was undertaken to automate the nicotinic acid analysis in orange juice.

We chose a chromatographic approach for the analysis. The sample hydrolysis step was based on the AOAC method (30 min. in the autoclave at 15 psi with 1N H_2SO_4). The hydrolyzed sample was dialyzed automatically and the dialysate pumped through a short column of cation exchange resin by a Technicon proportioning pump. Nicotinic acid was resolved on the column from the interfering substances by means of a dilute sodium phosphate buffer. The column effluent was mixed first with cyanogen bromide and then followed by the addition of sulfanilic acid. The nicotinic acid reacted with these reagents as in the standard vitamin assay to produce a yellow color. The reaction mixture then passed through a flow-cell colorimeter and was recorded. The method combines the labor-saving advantage and improved precision of automation, the separation capability of column chromatography, and the specificity of a chemical reaction for quantitative read-out.

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*Work supported in part by the Lemon Products Technical Committee.

BYPRODUCTS FROM CITRUS PEEL EMULSIONS

William L. Bryan and Robert E. Berry
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

Recovery of other byproducts from citrus peel emulsions in addition to cold-pressed oil would facilitate waste disposal and reduce cost. Following work at our laboratory, distilled oil (> 95% d-limonene) is being recovered in many citrus processing plants by steam distilling the waste effluent from peel oil separators or the peel emulsion from juice extractors. The steam-stripping system is an important factor in achieving high recovery of oil with desirable aroma using minimum steam. Second, by means of a fixed-bed adsorption process water-soluble flavoring components can be concentrated and recovered from the aqueous co-distillate from steam stripping. Finally, the oil-free waste emulsion from steam distillation has potential value as a cloud and flavor additive for juice drinks and beverages.

Comparing efficiency of three basic stripping processes at the same steam consumption, oil recovery increased with degree of turbulence of the steam-emulsion mixture and was higher with co-current than counter-current flow. The processes compared were: (1) high temperature flash-flow expansion of superheated (250°F) emulsion through a one-quarter inch i.d. by 30 ft. tube into a vapor-liquid separator, which was the most efficient process; (2) co-current flow of steam and emulsion at atmospheric pressure in a vertical 2-inch i.d. by 7 ft. pipe with a vapor-liquid separator at the top; and (3) counter-current flow at atmospheric pressure in a 4-inch i.d. by 3-ft. column with baffles to promote vapor-liquid contact.

A commerically feasible adsorption process to recover valuable flavoring components from the aqueous co-distillate from steam stripping Valencia orange emulsion has been demonstrated. Amberlite XAD-4 polymeric adsorbant in a fixed bed column (length/diameter ratio = 12) adsorbed up to 20% of its dry weight of organic components from 800 bed volumes of aqueous distillate (100 ppm recoverable compounds). Bed capacity was determined from breakthrough curves of effluent concentration, and the column was operated until linalool in the effluent increased to 10% of feed concentration. Breakthrough concentration could be detected by UV monitoring the effluent as well as adsorbent color change. The capacity of the polymeric adsorbent for individual compounds increased markedly with increasing molecular weight, and most compounds with fewer than seven carbon atoms were not recovered. Compounds were eluted from the saturated adsorbent bed as a 3% solution in ethanol. In flavor tests this mixture of recovered compounds could not be distinguished from a 90-fold terpeneless concentrate prepared from Valencia cold-pressed oil by extraction and steam distillation.

De-oiled citrus emulsions are potentially valuable as natural clouding agents for the drink and beverage market, especially in Europe, where peel-like character is desired. Initial cloud and cloud stability during extended centrifuging were similar to fruit juices and suspensions recovered from counter-current washing of pulp from juice finishers. A 1.8° Brix Valencia orange de-oiled emulsion was centrifuged to remove large particles and concentrated 20-fold. Up to 80% of the original cloud could be regained after reconstituting, and freezing the concentrate had no harmful effects. Reconstituted emulsion retained some characteristic peel aroma and was bitter.

These three byproducts are available in large enough quantities to have a significant impact on citrus processing economics. For example, potential steam-distilled d-limonene production in Florida alone could approach 70 million lbs annually, while recovery of flavor compounds by adsorption from aqueous co-distillate could reach 30,000 lb/yr. Production of de-oiled peel emulsion could approach 7 billion lbs/yr.

ENZYME CHEMISTRY OF LIMONIN

I. METABOLISM OF LIMONOIDS IN CITRUS

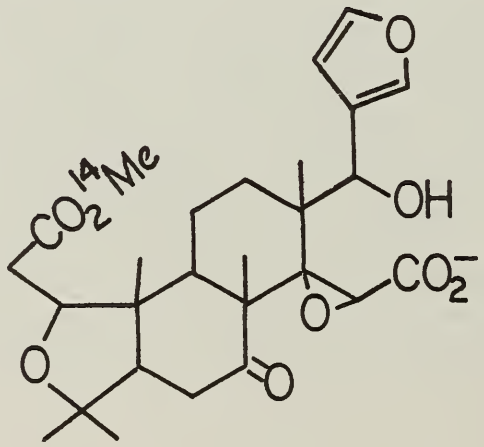
Shin Hasegawa, V. P. Maier, and R. D. Bennett
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Limonin is an intensely bitter tetracyclic triterpenoid dilactone, which is responsible for bitterness in some citrus processed products. Considerable research has been done on limonoid chemistry, but the biochemistry of limonoids is just beginning to be understood.

During the past 3 years, considerable progress has been made on the metabolism of limonoids in bacteria. We now know that limonoate A-ring lactone, the naturally occurring form of limonin in citrus fruit, is metabolized in bacteria through two pathways; one is via 17-dehydrolimonoate A-ring lactone, and the other is via deoxylimonin.

However, our knowledge of limonoid metabolism in plants is very limited. Limonin D-ring lactone hydrolase is the only limonoid specific enzyme that has been isolated from citrus. The limonoid content of citrus fruits is also known to decrease with advancing maturity and during postharvest storage. Also, postharvest exposure of fruit to low levels of ethylene has been shown to significantly accelerate limonoid metabolism. For many years, attempts to elaborate the natural limonoid-degrading system of the fruit have not been successful.

In a new approach to this problem, the metabolism of a structurally related limonoid was demonstrated in tissue slices of navel orange albedo. When incubated with tissue slices, 19-deoxylimonoic acid 3-methyl-^{14}C ester (see figure) was converted to 17-dehydro-19-deoxylimonoic acid 3-methyl-^{14}C ester and one other unidentified compound. These results, coupled with the



fact that 17-dehydrolimonoate A-ring lactone is a natural constituent of citrus, show that the fruit possesses a limonoate dehydrogenase, and that one pathway of metabolism of limonoate A-ring lactone is via 17-dehydrolimonoate A-ring lactone. Whether limonoid metabolism in citrus also proceeds via a deoxylimonin-deoxylimonic acid pathway remains to be determined.

It is of interest to note that one of the first reactions of the natural metabolic pathway(s) of limonoids in citrus fruits is the same reaction catalyzed by the bacterial limonoate dehydrogenases currently being developed for commercial debittering of citrus juices.

II. DEBITTERING CITRUS JUICES WITH LIMONOATE DEHYDROGENASE ENZYMES

Linda C. Brewster, Shin Hasegawa, and V. P. Maier
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The search for microbial enzymes that degrade limonin has resulted in the isolation of two different limonoate dehydrogenases. These enzymes catalyze the conversion of the limonin precursor, limonoate A-ring lactone (LARL), to nonbitter 17-dehydrolimonoate A-ring lactone (17-DLARL) thereby preventing the conversion of LARL to bitter limonin in acidic solutions. The first enzyme isolated was from the bacterium Arthrobacter globiformis. More recently, a second limonoate dehydrogenase was isolated from another bacterium, No. 321-18. These enzymes are distinctly different proteins and have markedly different properties in model systems including the pH of optimal activity and cofactor requirements. Details of these properties and some juice treatment studies with the dehydrogenase of A. globiformis were reported at previous Conferences.

Preliminary attempts to directly debitter navel orange juice using the new limonoate dehydrogenase were very encouraging. Consequently, a detailed study of the action and effectiveness of the new dehydrogenase in orange juice was undertaken. We found that the enzyme functions well in freshly prepared navel orange juices at their natural pH's. Sufficient native cofactors(NAD and NADP) are present in the juice to allow substantial conversion of LARL to nonbitter 17-DLARL. However, the addition of the cofactor NADP enhances enzyme activity and allows more efficient use of the enzyme.

The time needed for substantial debittering of freshly prepared navel orange juice was investigated. Substantial conversion was achieved in 15 minutes at 65°F using a moderate amount of enzyme. After 2 hours, no further conversion occurred. The fact that substantial conversion is achieved in a short time is important because it demonstrates that the enzyme-catalyzed conversion of LARL to 17-DLARL is rapid enough to effectively compete with the conversion of LARL to limonin.

Noting the success with the limonoate dehydrogenase of No. 321-18 in juice systems at their natural pH, the limonoate dehydrogenase of A. globiformis was reexamined. We found that it, too, would catalyze the debittering conversion of LARL to 17-DLARL in fresh navel orange juice at its natural pH provided substantial quantities of enzyme and cofactor were added.

This discovery led us to study the stabilities of the two enzymes, especially in the low pH range encountered in citrus juices. The limonoate dehydrogenase of No. 321-18 retains much of its activity after incubation at pH 3.5, has its maximum stability at pH 6.5, and its stability decreases as the pH increases above pH 6.5. In contrast, the limonoate dehydrogenase of A. globiformis retains only minimal activity at pH 3.5, but is quite stable over a broad range at high pH levels (pH 6.0-9.5).

Considering the above stability data, the limonoate dehydrogenase of No. 321-18 appears much more suitable for the direct debittering of fresh, low pH citrus juices than does the enzyme from A. globiformis. At higher pH, the limonoate dehydrogenase of A. globiformis is quite stable, making it potentially useful for the debittering of other citrus tissue systems having higher natural pH values than juice. It has been shown to be effective in reducing the eventual limonin content of both an orange peel slurry and a cloudy lemon seed suspension. Debittering might allow more extensive use of these tissues in products and byproducts such as flavoring, coloring and clouding agents and comminuted whole fruit or peel products.

STUDY OF BINDING OF LIMONOIDS TO PROTEINS BY NMR AND FLUORESCENCE SPECTROSCOPY

Raymond D. Bennett
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Some of the most fundamental biochemical processes involve specific binding of small molecules to proteins. Such phenomena include enzyme-coenzyme, enzyme-substrate, antibody-antigen, and receptor-hormone interactions. Also, compounds having tastes or odors probably produce their characteristic effects by binding to specific receptor proteins in the tongue or nasal passages. Investigations into the nature of these interactions at the molecular level have only become possible within the past few years as sophisticated spectroscopic methods have become available. Two of these techniques, nuclear magnetic resonance (NMR) spectroscopy and fluorescence spectroscopy, have now been applied to study binding of some bitter citrus limonoids to proteins.

An NMR spectrum shows signals for each of the protons in a compound, and the spectral changes observed upon interaction with a protein can thus indicate which parts of the molecule are involved in binding. The signals of protons near the binding site are always broadened, and in some cases shifts in position of the signals are also observed. Thus, NMR can be a powerful tool for probing such molecular interactions. Unfortunately, its lack of sensitivity is often a serious limitation. With conventional NMR instruments, measurements at concentrations much below 10^{-2} molar are not practical. Since protein binding studies must be done in water, this technique is only applicable to compounds that are relatively soluble in water. The water solubility of neutral limonoids is about 10^{-5} molar, so they can only be studied by opening a lactone ring to form a salt or by preparing water-soluble derivatives. Both approaches have been used in this work.

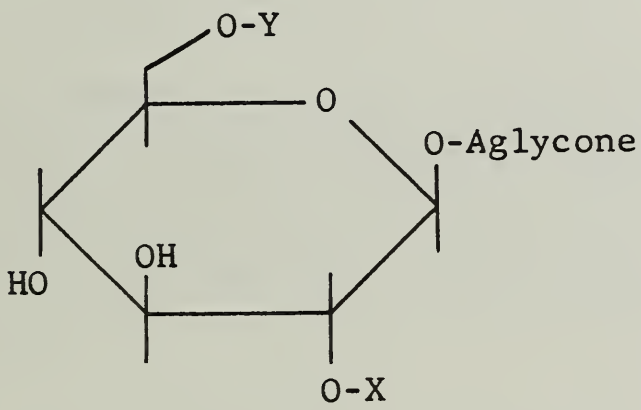
Because of the low sensitivity of the method, about 10-20 mg of protein are required for each spectrum. Many biologically important proteins, such as enzymes, cannot readily be obtained in such quantities. Therefore, bovine serum albumin (BSA), which can bind diverse types of compounds, has been used as a model protein to investigate the feasibility of studying limonoid-protein interactions by NMR. Sodium limonoate, produced by opening both lactone rings of limonin, and limonin carboxymethoxime, a water-soluble derivative of limonin, both were shown to bind to BSA by NMR, and the functional groups involved in binding were determined. It is of some interest that limonin carboxymethoxime, which is comparable to limonin in bitterness, was tasteless in the presence of BSA.

In contrast to NMR, fluorescence spectroscopy has the advantage of very high sensitivity, the lower limit being in the region of 10^{-10} molar. Furthermore, fluorescence spectra are often dramatically changed upon binding of a fluorescent compound to a protein. Although these changes do not reveal the interaction sites in the molecule, they can provide considerable information about the size and shape of the protein. Limonoids are not naturally fluorescent, so it was necessary to prepare fluorescent derivatives. The binding of nonfluorescent limonoids to proteins could then be studied by competition experiments with the fluorescent derivatives. Some preliminary results of this approach will be discussed.

NEW TYPES OF STRUCTURAL MODIFICATION OF DIHYDROCHALCONES AND THEIR EFFECT ON TASTE

R. M. Horowitz and Bruno Gentili
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The sugar component of dihydrochalcone glycosides and flavanone glycosides is crucial in determining taste or tastelessness in these compounds. We showed earlier that if the sugar component is β-neohesperidosyl (2-O-α-L-rhamnosyl-β-D-glucosyl) (I), the taste of many dihydrochalcone derivatives is intensely sweet, whereas the taste of various flavanone derivatives is intensely bitter. When the 2-O-rhamnosyl substitutent is absent (II) the taste is usually the same as in the neohesperidoside, but of diminished

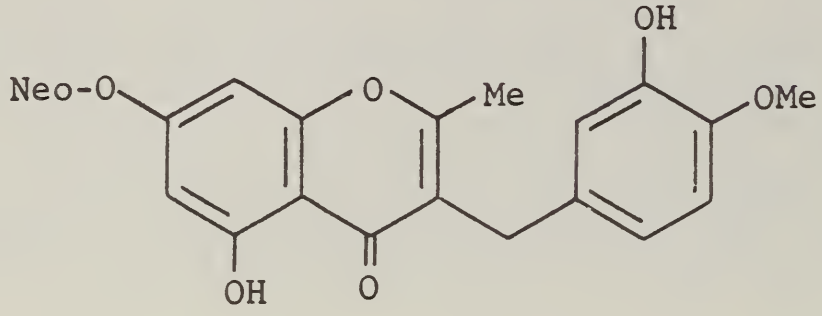


I: X = α-L-rhamnosyl; Y= H
II: X = Y = H
III: X = H; Y = α-L-rhamnosyl
IV: X = α-L-rhamnosyl; Y= Me; Aglycone = hesperetin dihydrochalcone
V: X = Y = α-L-rhamnosyl; Aglycone = hesperetin dihydrochalcone

intensity. Transposition of the rhamnosyl substitutent from the C-2 hydroxyl to the C-6 hydroxyl group to give β-rutinosyl (III) invariably results in abolishing the taste, regardless of the nature of the aglycone. On the other hand, the addition of a methyl group to the glucosyl C-6 hydroxyl of neohesperidin dihydrochalcone (IV) causes no measurable change in sweetness. In view of these results it was of interest to determine the effect of placing a rhamnosyl group at the glucosyl C-6 hydroxyl of neohesperidin dihydrochalcone. This trisaccharide (V) has the structural elements of both a neohesperidoside (which should be sweet) and a rutinoside (which should be tasteless). Compound V was synthesized in low yield from the 6"-O-trityl derivative of neohesperidin dihydrochalcone. It was obtained as a highly soluble crystalline

product whose structure was confirmed by NMR. V was sweet, but the sweetness was several orders of magnitude less than that of the parent compound. The conclusions that can be drawn from this result will be discussed.

A second new compound, the methyl chromone VI, was prepared from



VI

neohesperidin dihydrochalcone by condensation with acetic anhydride. VI is a crystalline substance and has surprisingly high solubility in water. Its taste, initially, is very slightly bitter, changing on drinking water to a barely detectable sweetness. When mixed with solutions of neohesperidin dihydrochalcone it markedly suppresses the initial sweetness, but seems to have somewhat less effect on the after-sweetness. The implications of these findings will be discussed.

DIHYDROCHALCONE SAFETY EVALUATION STUDY IN RATS

A. N. Booth, D. J. Robbins, M. R. Gumbmann, and D. H. Gould
Western Regional Research Center
Berkeley, California

The discussion will concern the results to date of a systematic toxicological evaluation of neohesperidin dihydrochalcone (NDHC), a potential noncaloric sweetening agent for use in foods. This includes a chronic rat-feeding trial in which dietary levels of NDHC up to 5% were fed to rats for 2 years. The conventional data recorded during the long-term study included body weights, mortality, feed consumption, hematology and urinalysis. Various biochemical analyses were performed on blood and liver samples obtained at termination. In addition, all surviving rats were autopsied and examined for evidences of gross pathology, and specific organ weights were recorded. Finally, numerous tissues were specially prepared and examined for the presence of microscopic lesions.

A multigeneration reproduction study was also conducted, including a teratology study, of the effects of NDHC. Work now in progress is concerned with (a) the metabolic fate of isotope labeled NDHC and (b) a long-term, dog-feeding trial.

Several supplementary studies to be discussed will include the effects of NDHC on thymus involution, on basal metabolic rate, on glucosuria, and on mutagenesis.

From the results obtained to date, it was established that at the highest dietary level fed (5%), positive effects were produced in rats, including a reduction in weight gains, and higher liver, kidney, and thyroid weights. All of these effects could be counteracted by modification of the basal diet to which the NDHC was added. Pending completion of those phases of the toxicology investigation still in progress, it is tentatively concluded that the proposed use of NDHC as a food additive continues to look promising.

PESTICIDE RESIDUE REDUCTION BY THE PROCESS OF PREPARING WHOLE ORANGE PUREE

Roger F. Albach and Bruce J. Lime
Subtropical Texas Area
Food Crops Utilization Unit
Weslaco, Texas

The puree-process developed by Cruse and Lime of our laboratory incorporates 85 to 90% of the entire fruit into the final product. An essential, initial step of their process is a 10-minute hot-water blanch of the whole fruit prior to grinding. This blanch along with subsequent removal of seeds and hard portions of tissue in the finisher would be expected to eliminate a considerable amount of pesticide residue initially present in the field-run fruit.

In this study we determine what effect the whole citrus fruit puree process has on the content of pesticide residues in the final product and simultaneously determine the probability of pesticide residues exceeding established tolerance limits on fruit used in the preparation of purees.

The study was divided into two parts: The first involved exposing trees bearing mature fruit to a mixture of nine pesticides and then harvesting the fruit after various time intervals following exposure. The aim here was to obtain mature fruit which had been subjected to what would constitute a grossly negligent, uneconomical and illegal application of pesticides immediately prior to harvest.

The second part of the study was to obtain mature fruit from trees which had undergone a documented normal pesticide program during the season prior to harvest.

In both parts of the study the samples were analyzed for pesticide residues at stages in the post-harvest handling and processing. At each collection date six samples were divided into three post-harvest treatments of two replications each. The three treatments were:

Unwashed - the fruit prepared for analysis as taken from the tree.
Washed - the fruit washed in a manner similar to that employed in commercial processing plants.
Pureed - the fruit washed as above then made into whole orange puree by the published method of Cruse and Lime.

In no case did any individual pesticide exceed the tolerance limits established by the U. S. Environmental Protection Agency. This was true even of unwashed fruit harvested the day immediately after spraying.

Simple washing of the fruit removed from 8 to 35% of the residue initially present on the unwashed fruit. The puree process in addition to the wash resulted in a 71 to 95% reduction in residues.

Even under the extreme conditions of this experiment the puree was never found to contain more than 6% of the EPA tolerance limits established for raw fresh citrus fruits.

When one considers that harvesting fruit without waiting a prescribed time after pesticide application is contrary to good grove management and statutory regulations, it can be tentatively concluded from the foregoing results that it is improbable that fruit reaching the processor would exceed EPA tolerances and therefore pesticide residues in whole citrus fruit puree is unlikely to constitute a health hazard to the consumer.

Such a conclusion is supported by the results of the second part of the investigation where no greater than 1.4% of the EPA tolerance limits were found in the samples from fruit receiving a normal spray program and harvested in early January.

ENHANCEMENT OF COLOR AND PROVITAMIN A QUALITY OF CITRUS FRUIT*

I: NATURE OF INVESTIGATIONS ON CAROTENOID PIGMENT INDUCERS.

H. Yokoyama, W. J. Hsu, S. M. Poling, and C. DeBenedict
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

At this meeting last year, we showed that carotenoid color and provitamin A formation in oranges and other citrus fruits can be regulated. At that time, we reported on a number of chemical compounds, 2-(4-chlorophenylthio) (CPTA) and chalcone derivatives of triethylamine, which caused a net synthesis of provitamin A carotenes and, in addition, a rapid and large accumulation of the intensely red pigment lycopene. This latter effect resulted in an accelerated change of color from orange through deep orange to red, for example, in oranges. This effect is potentially useful for improving the color of fruit intended for processing because color development can be halted at the desirable deep-orange color by juicing the fruit. However, it is unsuitable for fruits destined for the fresh fruit market.

In continuing our studies on the development of methods for enhancing the natural color and provitamin A content of oranges and other citrus fruits, we have been concerned with the design and synthesis of new and better color inducers. These efforts, in the past year, have led to the discovery of new improved color and provitamin A responses in citrus, namely (1) the retention of the deep-orange color for much longer periods of time; (2) increased production of the provitamin A α-, β-, and γ-carotenes; and (3) induction of only deep-orange-colored carotenoid pigments. These observed, highly desirable responses will be described more fully in the following two papers. These discoveries represent a major step forward in the search for chemical compounds that selectively induce desirable carotenoids and responses.

In addition to the chemically synthesized inducers, we have been investigating carotenoid color inducers isolated from natural sources. Initial studies have been conducted on these natural inducers using the relatively simple biological system found in the carotenogenic mold Blakeslea trispora. Several of these naturally occurring inducers have shown activity of as high as a 10-fold increase in β-carotene production in the mold. These effects and their implications will be discussed.

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*Supported in part by the California Citrus Advisory Board.

II: STUDIES ON THE STRUCTURE AND ACTIVITY OF SOME NEW SYNTHETIC INDUCERS.

S. M. Poling, W. J. Hsu, and H. Yokoyama
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The ability of CPTA and chalcone derivatives of triethylamine to enhance the natural color and provitamin A content of citrus, including the navel orange, the Marsh seedless grapefruit, and the trigeneric citrus hybrid Sinton citrangequat, has been previously reported. Five new synthetic compounds, also causing an increase in the carotenoid pigment formation in citrus, are reported. The compounds, namely [β-(diethylamino)-ethoxy]-benzene (I), [γ-(diethylamino)-propoxy]-benzene (II), [δ-(diethylamino)-butoxy]-benzene (III), 4-[β-(diethylamino)-ethoxy]-benzaldehyde (IV), and diethylaminoethyl anisolate (V), caused a 5- to 12-fold increase in the pigment content.

The Marsh seedless grapefruit was used in these tests because of the relative simplicity of the carotenoid pattern and the ease of detecting any color changes visually. The compounds are as effective on oranges. In contrast to previous work with CPTA, where the fruit was treated by short periods of immersion in aqueous solutions, compounds I-V were applied by using a solution of the free amine in isopropanol. This gave a much more uniform response in the color development.

Examination of the flavedo of the treated fruit showed that lycopene accumulated as the major pigment in each case. There was also a significant increase in the amount of ζ-carotene. The response pattern of the treated fruit is similar to that of fruit treated with CPTA. CPTA and compounds I-V can all be considered as derivatives of triethylamine.

Fruit treated with CPTA rapidly change from the natural yellow color to pink or red due to the lycopene accumulation. In fruit treated with the new compounds, the development of color (depending also on the concentration) is much slower. The fruit change slowly from yellow through light to deep orange, and finally to red due to lycopene; the treated fruit retain the desired deep-orange color for a much longer time (3-5 weeks at room temperature).

These new compounds show potential usefulness in bringing about desirable color changes in citrus fruits.

III. TWO NEW CLASSES OF INDUCER RESPONSES

W. J. Hsu, S. M. Poling, and H. Yokoyama
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Previously, it was shown that CPTA and chalcone derivatives of triethylamine caused the intensely red-colored lycopene to accumulate as the principal pigment, and as a result the fruit developed a deep red color. In studies

conducted in the past year, we have discovered a group of new chemical color inducers that produces orange-colored fruit only. These color inducers are the first ones observed that cause a substantial increase in carotenoid pigment formation in citrus, including oranges, without stimulating the large accumulation of the red pigment lycopene. The deep-orange color produced by these inducers is due primarily to the formation of two, as yet unidentified, carotenoids. The rate of fruit color development can be controlled by the concentration of the inducers applied; however, the color of the treated fruit does not go beyond the deep-orange level. The discovery of this class of inducers has shown that the formation of carotenoids possessing the desired intensity of color (deep orange) for citrus can be induced and controlled. Specifically, this class of inducers is potentially useful for improving the color of fresh market oranges.

Also within the past year, another group of color inducers was discovered at this laboratory. This group of compounds causes citrus fruits to produce three provitamin A carotenoids (α-, β-, and γ-carotenes) as well as lycopene. Fruit color development proceeds rapidly to deep orange, then continues into red. However, in all cases, the desired color (deep orange) can be retained much longer (2-3 months) by storing the fruit at lower temperatures (ca. 4-10°C or ca. 40-50°F). In general, when color development occurs, it is accompanied by a 2- to 3-fold increase in the provitamin A carotenoids. This group of inducers is potentially useful for improving the color of fruit intended for both processing and fresh marketing.

LEMON JUICE CLOUD; SOME EFFECTS OF PROCESSING*

A. W. Venolia, S. A. Peak, F. W. Payne, and K. Nelson
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Work on the particulate matter of lemon juice was continued along the lines discussed last year. New unpasteurized juice samples as well as laboratory and commercially processed samples were evaluated in terms of particle-size distribution and juice turbidity. The variety of unpasteurized samples examined was sufficient to provide clues concerning juice variability that may be encountered in the future. The work with unpasteurized material has also provided estimates of experimental precision needed for interpreting test results.

Laboratory juice processing was a very mild and simplified version of commercial practice. While the results of laboratory processing were, therefore, not directly comparable with plant processing, the contrasts encountered illuminate, in a number of ways, the property changes found in the commercial samples.

Laboratory processing did not include an analog of the paddle finishing operation of the plant; this finishing operation increased the concentration of detectable particles and turbidity index (12 and 31%, respectively) while it decreased modal particle diameter and distribution width (7 and 6%, respectively). The effect of the finishing operation can be summarized by observing that it increased the light-scattering efficiency (ratio of turbidity index to concentration of detectable particles) 18%.

While effects attributable to pasteurization were comparatively slight, the production of juice concentrate caused substantial changes both in the plant and in the laboratory. Although both concentration operations decreased modal particle diameters and distribution widths, laboratory concentrate had a larger modal particle diameter and a wider particle size distribution. Concentration in the plant had no net effect on light scattering efficiency, but laboratory concentration raised it 36%.

The relatively large hydraulic shear forces generated in equipment such as juice finishers and centrifugal pumps were largely absent during laboratory processing. Suitable allowance must be made for the effects of such factors in interpreting the experimental results.

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*Work supported in part by the Lemon Products Technical Committee.

LIST OF PUBLICATIONS AND PATENTS*

WESTERN REGION

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, California 91106

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